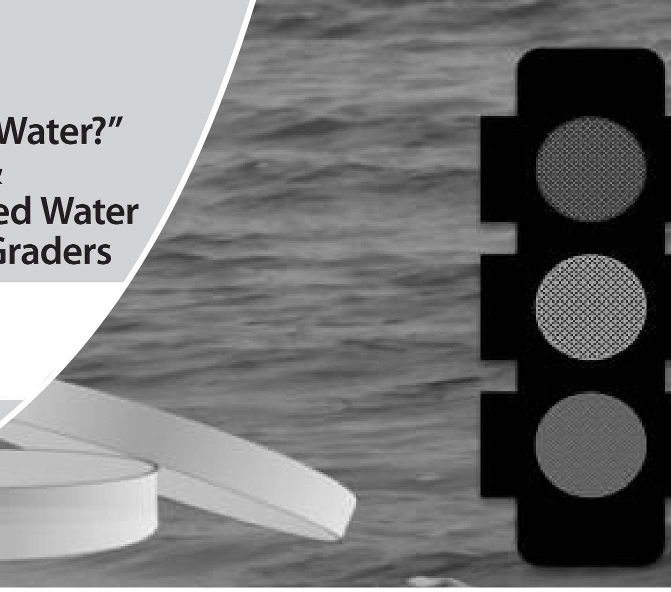

"STOP: Can You Drink That Water?" Microbiology, Chemistry, & Advocacy in an Inquiry-Based Water Quality Curriculum for 8th Graders

NABT BioClub RECOMMENDATION

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Abstract

For many middle school students, connections between their lives and concepts like chemical reactivity, microbial contamination, and experimental sampling are not obvious. They may also feel that, even if there were connections, understanding the monitoring and quality of natural resources is something for grown-ups and beyond their responsibility. This curriculum highlights connections. Students characterize bacteria in a local untreated water source and investigate the mechanism, effectiveness, and byproducts of chlorine bleach as a water treatment. Working in groups, they use different growth and treatment conditions to characterize samples, thus collectively obtaining a more complete description of the system. The North Carolina 8th Grade Standard Course of Study Objectives were used during curricular development, and alignment to Next Generation Science Standards performance expectations is provided. Teacher-guided discussions, demonstrations, experimentation, and database investigation engage students as they develop informed and critical opinions about water quality and water treatment methods. The final activity connects scientific investigation to advocacy and civic engagement.

Key Words: *NGSS; microbiology; chemistry; water quality; inquiry.*

○ Introduction

Increasingly, our world is shaped by scientific questions related to natural resources such as water, energy, and food. Often, however, students do not connect with these issues, perceiving them as too "far away" to maintain interest. Here, we present a curriculum that bridges the gap between classroom science and students' lives by using chemistry and microbiology to investigate the quality of local water sources. Students characterize the bacteria within a local water source, explore chemical reactivity and the practical aspects of water purification, discuss the ramifications of killing bacterial contaminants and accumulating the bacterial debris and reaction products, and learn about resources for advocacy and policy. The curriculum was designed according to education standards in North Carolina (Table 1) and matches multiple performance expectations in the *Next Generation Science Standards* (NGSS; Table 2). The NGSS emphasize an interdisciplinary approach to science education, and this curriculum exemplifies that approach by integrating natural science with intentional discussions of how science can inform real-world decisions and students' personal connections to natural-resource policy and advocacy.

Often, however, students do not connect with these issues, perceiving them as too "far away" to maintain interest.

We named the curriculum "STOP: Can You Drink That Water?" to highlight that we are all responsible for being aware of the quality and safety of our water and because it uses agars whose colors are the same as those in a traffic light. The curriculum's guided inquiry-based approach models the way in which scientists collaborate by requiring students to analyze their own data and pull together results from the whole class in order to draw a clearer picture of why water purification is needed and what it does.

The curriculum is flexible and affordable. If time doesn't permit the entire series, segments can be used individually. Additionally, to minimize costs, the exercises use household items wherever possible. Because it connects science, daily life, and society and encourages students to become engaged citizens, we wanted to ensure that the planned accessibility held true in practice. Therefore, we field tested the curriculum and found that it was successful in both a well-equipped school and a community-center summer program. Although this showed us that the curriculum could be integrated into a variety of teaching environments, it is intended for a science classroom.

○ Materials & Preparation

General

- Jar for collecting lake water
- Eyedroppers (2 per group); plastic transfer pipets can also be used
- Velcro tape strips (fabric/craft store)

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Table 1. North Carolina Standard Course of Study Objectives for 8th Grade Science addressed by the "STOP" curriculum.

Essential Standards, 6–8 Science	How Curriculum Addresses Standard
Overarching Standard: Science as Inquiry	Exercises include brainstorming, collecting and combining novel data, and revisiting the topic based on new findings.
8.E.1 Understanding the hydrosphere and the impact of humans on local systems and the effects of the hydrosphere on humans • 8.E.1.3 Predict the safety and potablity of water supplies in North Carolina based on physical and biological factors • 8.E.1.4 Conclude that the good health of humans requires (Monitoring of the hydrosphere/ Water quality standards/ Methods of water treatment / Maintaining safe water quality/ Stewardship)	Analysis of microbial contents of a local body of water (e.g., Lake Norman, Davidson, NC) Detecting bacteria in water, methods for treating and purifying water, understanding the need for water quality advocacy
8.P.1 Understand the properties of matter and changes that occur when matter interacts in an open and closed container • 8.P1.3 Compare physical changes such as size, shape, and state to chemical changes that are the result of a chemical reaction to include changes in temperature, color, formation of a gas or precipitate	Studying chemical reactivity via reactivity activity and formation of byproducts Discussing suitability of bleach as a disinfectant vs. other technologies like UV sterilization
8.L.1 Understand the hazards caused by agents of disease that affect living organisms • 8.L.1.1 Summarize the basic characteristics of viruses, bacteria, fungi, and parasites relating to the spread, treatment, and prevention of disease.	Introducing microbiology Introducing how bacterially contaminated water can cause illness

North Carolina Standard Course of Study Objectives for 8th Grade Science from http://www.ncpublicschools.org/curriculum/science/scos/

- Bleach
- Gloves (drugstore version sufficient)
- Masking tape
- Absorbent paper for work area
- Sharpie markers
- Craft scraps (combination of fuzzy or fabric and plastic or wooden items)
- Small bags for craft scraps
- Small (3 oz.) paper cups (3 per group)
- Small bottles (2 per group)
- 15 qt./14 L plastic storage box with lid
- Disinfectant spray for cleanup

Microbial Culture

- LB agar (Fisher Scientific, BP1425)
- Eosin methylene blue (EMB) agar (BD, 211215)
- Simmons' citrate agar (BD, 211620)
- Petri dishes (Fisher Scientific, 08-747-14g)

Field tests of this curriculum used agar plates prepared on site, per manufacturer's instructions.

Prepoured plates can be obtained from Carolina Biological Supply Company (LB: no. 822022; EMB: no. 821522) and through Ward's or VWR.com (Simmons' citrate: no. 89014-138). Sterile plates can be prepared and refrigerated for up to 1 month. Before use, the plates should be warmed to room temperature. To minimize condensation dripping on samples, plates are stored with the agar-containing side on the top ("upside down"). A water sample from a local lake, pond, or stream should be collected within 2 days of the plating experiment on Day 2. The PowerPoint lectures and handouts mentioned in the text, which include the required dilutions, are available at **http://www.bio.davidson.edu/people/kabernd/watercurric/index.html**.

○ "STOP: Can You Drink That Water?": Day 1

Why Don't We Just Drink Lake Water?

To begin the curriculum, students use a local lens to explore microbiology and methods of water treatment by discussing where their water comes from and what happens to the water before it reaches their tap. All content is delivered in an interactive format (outlined below), with information and questions used to lead discussion provided via PowerPoint (Presentation 1, online). Basic microbiology, illnesses caused by water-borne bacteria, and methods of water treatment are among the concepts covered.

Discussion Structure for Day 1

Brainstorm: Where do you get your drinking water? Why don't you get it from the nearest body of water?
Content delivery: Introduction to bacteria; impacts of bacteria on ecosystem function and human health.

Brainstorm: How can you detect bacteria in water? What would you need to detect bacteria?

Table 2. *Next Generation Science Standards* Middle School Performance Expectations relevant to the "STOP" curriculum.

NGSS Performance Expectations Middle school students who demonstrate understanding can:	Relevant "STOP" Curricular Elements
MS-PS1-2 Analyze and interpret data on the properties of substances before and after the substances interact to determine if a chemical reaction has occurred. *(Matter and Its Interactions)**	Students learn about structure and function of chemicals, including chlorine disinfectants, and apply knowledge in discussions of "reactivity activity" that simulates reactions and in interpretation of the byproduct-formation demonstration.
MS-LS1-1 Conduct an investigation to provide evidence that living things are made of cells; either one cell or many different numbers and types of cells. *(From Molecules to Organisms: Structure and Processes)* MS-LS1-2 Develop and use a model to describe the function of a cell as a whole and ways parts of cells contribute to the function. *(From Molecules to Organisms: Structure and Processes)*	Students learn about structure and function of bacterial cells and apply the information when • observing colony growth as a proxy for determining bacterial population in a sample and calculating the prevalence and abundance of bacterial species in the original samples and • interpreting experimental data that rely on the cellular composition of distinct bacterial species, allowing differential grow under selective conditions.
MS-LS1-5 Construct a scientific explanation based on evidence for how environmental and genetic factors influence the growth of organisms *(From Molecules to Organisms: Structure and Processes)* MS-LS2-1 Analyze and interpret data to provide evidence for the effects of resource availability on organisms and populations of organisms in an ecosystem. *(Ecosystems: Interactions, Energy, and Dynamics)* MS-LS2-4 Construct an argument supported by empirical evidence that changes to physical or biological components of an ecosystem affect populations *(Ecosystems: Interactions, Energy, and Dynamics)*	Students investigate how exposing mixed populations of cells to specific environmental conditions (three types of selective media and a series of bleach treatments) result in expansion of some populations and the death of others.

MS-LS2-5 Evaluate competing design solutions for maintaining biodiversity and ecosystem services, e.g. water purification. *(Ecosystems: Interactions, Energy, and Dynamics)*
 	
MS-ESS3-3 Apply scientific principles to design a method for monitoring and minimizing a human impact on the environment. *(Earth and Human Activity)*
 	
MS-ETS1-3 Analyze data from tests to determine similarities and differences among several design solutions to identify the best characteristics of each that can be combined into a new solution to better meet the criteria for success. *(Engineering Design)*
	• Students explore methods, effectiveness, and availability of water treatment techniques, in particular those used to treat their drinking water.
	• Students test and critique the pros and cons of using
	  • different sample dilutions to quantify microbes in water,
	  • different selective media to identify the microbes present, and
	  • different concentrations of chlorine bleach to kill microbes within a water sample,
	in order to develop a process that considers the usefulness of the information gathered and the effect that excessive bleach treatment would have on byproduct formation and human health.

Practices and Performance expectations from http://www.nextgenscience.org/search-performance-expectations?tid_2%5B%5D=14

*Parenthetical phrase contains NGSS Disciplinary Practice.

Content delivery: Introduction to use of plating water to determine bacterial content and the benefits of using various types of selective media.

Brainstorm: How could *you* remove bacteria from water?

Content delivery: How does disinfection work? Chemistry of bleach as a disinfectant; other methods of disinfection (boiling, UV light sterilization); methods used by water-treatment plants, including steps of coagulation, flocculation, sedimentation, filtration, and disinfection.

Figure 1. Gloves and examples of materials that can serve as "reactants" in the reactivity activity.

Optional Reactivity Activity

The highly reactive nature of chlorine bleach makes it an effective water treatment. Because this curriculum may be used before chemical reactivity has been discussed, we designed an "introduction to reactivity" activity. The activity can be conducted with full class participation or as a demonstration. A "Reactivity Worksheet" is found in the online materials.

To begin, students are provided the definition of a chemical reaction, an activity handout, bags of separate types of craft items (e.g., plastic beads, feathers, yarn, felt; Figure 1), and two plastic gloves. Glove 1 has "soft side" Velcro tape on its palm and fingers, and glove 2 has "sticky side" Velcro tape on its palm and fingers. The gloves are instant attention-getters. In this exercise, something sticking to a glove means that a "reaction" has occurred. Students reach into a bag, squeeze the material, and record what happens. They then repeat the process with the other bags of craft items. The importance of recording what they do and see should be stressed.

After students explore each of the bags, the instructor leads a discussion of which glove or craft items were more "reactive." Because the handout does not indicate whether "products" should be removed or allowed to accumulate between bags, groups may complete the exercise differently. This is part of the exercise. Using less prescriptive instructions allows the follow-up discussion to explore the analogy for reactivity *and* how differences between groups' approaches affect the information they gathered, underscoring the importance of good note taking and communication.

During field testing, students quickly saw that properties of the "reactants" determine how they interact and why testing bags in a different order or deciding to leave or remove previously attached items changed a glove's ability to react with subsequent items. The instructor's role is to connect the Velcro analogy to chemical reactions and reinforce that the properties of molecules determine how reactive they are (i.e., form follows function). Asking students to decide which craft-item reactant is most analogous to the highly reactive compound, bleach, connects the reactivity activity to earlier brainstorming about water treatment.

○ Day 2: What's in Your Water?

After discussing bacteria and drinking-water disinfection, students apply the knowledge in two plating experiments: one characterizing the microbial contents of lake water using selective media, and another exploring the effectiveness of disinfection. Students work in groups of fewer than four to ensure that each student has an active role (see "Water Testing and Treatment" worksheet online). In the activity, described in greater detail online, students plate water collected from the nearest body of water onto three growth agars: EMB (red), LB (yellow), and Simmons' citrate (green). The type of bacteria that will be able to grow is determined by the medium's components. LB is not selective and allows growth of many aerobic bacteria strains. EMB allows growth of Gram negative bacteria (such as *E. coli*) but not Gram positive ones. Simmons' citrate agar allows growth of bacteria that can use citrate as a sugar source. The media used in your classroom can vary, depending on what is available to prepare or order.

If possible, different types of selective media should be used so that students can discover how plating on different types of media can provide more detailed information about which bacteria are present and how that information might indicate causal events in the body of water sampled (e.g., high numbers of colonies on LB and EMB suggest *E. coli* and could indicate a sewage spill upstream).

In the first plating session, each group is given one of each type of selective media plate and lake water that is either "full strength" or diluted with tap water (instructions online). Wearing gloves and using eyedroppers, students add 20 drops of lake water to each plate and swirl to cover the agar surface. During data analysis, students will discover that dilutions are necessary because the body of water used will affect the number and types of microbes present. Some concentrations of lake water may produce too many colonies to count, whereas others do not contain bacteria that grow in a certain condition, each result providing important information about the bacterial population in the sample. Having groups test different dilutions reduces cost because it allows the class to have interpretable data without every group using the materials needed to perform serial dilutions. Because the groups must combine data in order to interpret the system they are studying, the investigation models how scientists collaborate to get a more complete and interpretable picture.

Plating different dilutions also allows discussion of the mathematical manipulations needed to (1) see if the colony counts are consistent across dilutions and (2) estimate the number of bacteria in the original lake water (the colony-forming units, or CFU). Depending on the goals of your course, the number of colonies resulting from different dilutions of the same sample can prompt a conversation about sampling techniques, probability, sample size, and the practical consideration of wanting to have enough colonies to be significant, but not so many that the data set becomes unwieldy to count. In addition, classes with greater than four groups will have multiple groups testing a particular dilution. This allows discussion of why the data were or were not repeatable and the importance of replicates.

In the second plating session, the effectiveness of disinfection on subsets of bacteria is investigated; everyone works with "full strength" lake water, but each group has only one selective medium. Groups are provided three plates, bleach, three paper cups, and eyedroppers specifically marked "bleach only." They label the cups "1/5," "1/10," and "1/20," and samples are prepared by adding first lake water and then drops of bleach to each cup. (Note: The order is important. If bleach is added first, the initial drops of lake water will hit full-strength bleach and all bacteria will be killed.) After gently swirling to mix the components, students plate samples onto their selective plates.

In preparation for the growth period, every plate's lid is taped to minimize accidental contact with microbes, and the plates are stored in a plastic box to contain odors. At room temperature, colony formation requires ~48 hours. If a 37°C incubator is available, colonies should be visible after ~24 hours. If exercise days need to be spread farther apart, after colonies are visible the box can be moved to a laboratory refrigerator and stored for multiple weeks at 4°C (do not store with food, and do not freeze).

After plating the samples, students complete a think–pair–share and predict:

- What do the different types of media allow to grow and what do they keep from growing? Why or how do they allow this selective growth?
- Which plates will show the most bacterial growth?
- Which plates will have the least bacterial growth?
- If nothing grows on the citrate plates, what can you conclude about the population of bacteria in that sample?
- How does bleach kill bacteria?
- Which concentration of bleach will kill the most bacteria, and which concentration the fewest?

Encourage students to express predictions as statements that include "Because…" or "Since…" phrases and provide support for their assertions. You can then reinforce that these are the class's hypotheses and review the important components of hypotheses: that they are based on previous information and are testable and falsifiable. Students should record these hypotheses so that they can refer to them during data interpretation and when communicating the study's conclusions.

○ Day 3

Analysis of Microbial Presence, Diversity, & Disinfection

After reviewing that a colony is not one large cell but millions of offspring of a single plated cell, students count and describe the different types of colonies on each plate. The group data are pooled, and the class discusses what colony growth on different types of media says about the bacterial population in the water sample and which concentration of bleach was sufficient to "purify" the water (i.e., to kill the bacteria below detectable levels). If cameras or cell phones are available, students can collect images for analysis at another time or to use as part of a presentation extension assignment.

These discussions most effectively model scientific practices if the hypotheses developed on Day 2 are briefly reviewed and the analysis focuses on which ones are supported by data and which are not. The underlying message must be that data are not discarded or considered "wrong" if they do not support a hypothesis. The interpretation must fit the data collected, not the other way around ("Data Collection and Analysis Worksheet" online).

Exploration of Disinfection Byproducts & American Drinking-Water Quality

Equipped with knowledge about water pollution and water treatment and experimental evidence of the microbial diversity within their own local untreated water source, students now investigate what can happen to water as it travels from a treatment facility to houses. This section of the curriculum focuses on the formation of byproducts and the nonbacterial contents of tap water.

Byproduct Demonstration

To introduce the concept of byproduct formation, we used a pH indicator dye and an apparatus consisting of a plastic funnel attached to a flexible tube and a transparent catch container. Indicator dyes can be purchased; however, the dye we field tested was the liquid left after boiling red cabbage. We recommend preparing this particular pH indicator in a fume hood or at home because boiled cabbage's distinctive odor will permeate school hallways. Fortunately, this indicator dye can be prepared and refrigerated for a month. The demonstration apparatus is easily prepared from materials available at local hardware and grocery stores or common laboratory equipment (Figure 2).

Before the demonstration, a cotton ball soaked with lemon juice *or* dilute NaOH is inserted into the tubing. Students are told that the funnel represents a water-treatment holding tank, the tube is the pipe leading to homes, the cotton ball represents all the things the treated water contains or interacts with (disinfection reaction products, cellular breakdown products after disinfection, and interactions with the pipes themselves), and the catch container is the faucet in their house. The freshly disinfected water from the treatment facility (cabbage juice) is then added to the funnel. As the purple-colored juice interacts with the cotton ball, it dramatically changes color (e.g., lemon juice = purple to pink, NaOH = purple to green). Asking leading questions helps guide the students to realize that even treated water is not "just" H_2O. Reactive elements left after treatment can interact with each other, causing side reactions and accumulation of byproducts as water travels to our faucets.

Figure 2. Byproduct demonstration setup. The purple-colored red cabbage pH indicator dye is added to the funnel. Traveling down the tubing, it interacts with a cotton ball soaked in NaOH, turns green, and drips into a catch container.

American Drinking-Water Quality

After the concept of byproducts is introduced, the class uses a PowerPoint presentation to explore water-purification byproducts and other possible components of tap water (Presentation 2, online). The PowerPoint contains interactive elements drawing from a *New York Times* study's data on contaminants in American tap water (http://projects.nytimes.com/toxic-waters/contaminants). If time and computers are available, however, this can be replaced with more student-driven research exploring the water quality in their hometown or region using the *New York Times* database. The curriculum ends with discussions of how students can make their voices heard to praise or express concern about their local water quality, and the importance of being aware of resource management issues that affect their lives and of advocating for policies that support healthy environments and people.

Discussion Structure for Day 3

Content delivery: Definition of "byproduct."
Brainstorm: Can you think of examples of byproducts? (Instructor alludes to the color-change demonstration and the chemistry lessons from Day 1, guiding students toward understanding that reactions can cause byproducts and that the products of one reaction [e.g., dead bacteria] may be reactants for additional side reactions.) Are there any highly reactive agents present in our tap water? *Yes, bleach!*
Content delivery: Overview of water-disinfectant byproduct formation and risks.

Investigate: Students explore the content of water systems across America, using data provided in a 2009 series produced by *The New York Times*. This series contains an interactive feature that allows users to browse data on the content of American water systems.
Content delivery: Overview of how to interpret the data.

Investigate: Students investigate types of contaminants found in five cities' water supplies: New York City, Idaho Falls, Honolulu, Charlotte, and Durham (PowerPoint 2). To facilitate discussion, examples of different types of contaminants (e.g., disinfectant byproducts, agricultural byproducts, plumbing byproducts) are indicated by circles of different colors.
Brainstorm: Why might some contaminants be present in higher levels in some water systems than in others? Example: There are more agricultural byproducts in Idaho Falls water than in New York City water. Why?
Content delivery: Instructor points out common contaminants and explains the implications of their presence. Note that most contaminants are not present at dangerous levels, but if they build to higher levels there may be a public health problem.

Brainstorm: Who can you contact if you have questions, praise, or complaints about your tap water?
Content delivery: Instructor explains the importance of being informed and involved, and how to find information and contacts for local treatment facilities and local and state representatives. Information is reinforced with a worksheet covering how students can stay informed about the safety of their tap water ("Making Your Voice Heard" handout).

○ Assessment

Example assessment questions are provided below. To preserve the integrity of the questions, full pre- and post-curriculum assessment tools are available by emailing the corresponding author. In the full materials, the assessment is broken into subcategories testing knowledge of bacteria, chemistry, water, and advocacy, so that each subcategory can be evaluated separately. During field testing, scores increased significantly between pre- and post-assessments in each of the subcategories ($P < 0.0002$).

Field testing included a combined 6th–9th grade Freedom School summer enrichment program (15 students) and multiple classes of 8th graders during the school year (4 classes/110 students). In all situations, teachers and students enthusiastically received the curriculum. Students remained engaged during the activities and expressed interest in learning more on their own. Although the disparate levels of background among the 15 Freedom School students confound interpretation of learning gains, the consistent engagement and enthusiasm of this group of 6th to 9th graders showed us that the curriculum could be used to introduce the topics covered.

The in-semester 8th-grade offerings included students and facilities that were the intended audience for this curriculum. After completing the curriculum, students demonstrated better use of topical vocabulary and could provide more nuanced explanations about the topics covered and the role of science and experimentation in their lives. As an example, one class's average score increased significantly, from 65.76% to 83.65%, between pre- and post-curriculum assessments ($n = 29$, $P < 0.0001$). No student's performance decreased on the post-assessment; in fact, the average individual post-assessment scores demonstrated a 28% increase over the paired pre-assessment scores. Because our program occurred after students had completed units on chemical reactions, the hydrosphere, and bacterial cells, the assessment data suggest that our water-quality curriculum reinforced and extended their previous knowledge and could also be used as a capstone activity.

Example Assessment Questions

What are bacteria? Circle all that apply:

Consumers	Eukaryotes	Producers
Decomposers	Germs	Prokaryotes
Dirt	Microorganisms	Viruses

Bacteria help humans. Circle the best answer:
Always true. Sometimes true. Never true. I don't know.
If you chose *always, sometimes*, or *never,* write a sentence to explain what you mean.

Why is bleach good at killing bacteria? Circle the best answer:
It's a strong acid. It's photosynthetic. It's highly reactive.
It's an antibiotic. I don't know.

If you have something to say about how good or bad your tap water's quality is, who can you contact and how?

Do you think you can use the scientific method in your everyday life?
Yes. No. I don't know. I don't know what the scientific method is.
If you chose *yes* or *no*, explain why.

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